

Protocol for ligation (10µl system)

Pipet the following into a 0.5ml microfuge tube:

Insert DNA	3:1 molar excess over vector
Linearized vector DNA	around 100ng
T4 DNA Ligase(TaKaRa)	1µl
10xT4 DNA Ligase Buffer(TaKaRa)	1µl
dd H ₂ O	rest of the volume

Vortex thoroughly and spin briefly;

Incubate the mixture at 16°C overnight;